Human Milk Glycans
David S. Newburg

The study by Grulee and colleagues of over 20,000 infants of 0-9 mo. age in Chicago from 1924-9 provided compelling evidence of the protective efficacy of human milk compared to either full or partial feeding with human milk substitutes. Among the data were striking mortality figures, showing ten times the rate of deaths among the artificially fed group than among the breast-fed infants. Compared with breastfeeding, the Grulee study indicates that artificial feeding confers an increased relative risk for morbidity of 3.1 fold and mortality of 7.1 fold. Additional benefits supplied by ideal nutrition extend beyond the immediate prevention of acute infectious disease.

Protective components of human colostrum and milk include prebiotic elements, sIgA, and multifunctional agents of the innate immune system. In addition, the ability of the oligosaccharides and glycoconjugates of human milk to protect the infant is now becoming more evident. This protection may be some of the strongest offered by human milk.

The many hundreds of distinct human milk oligosaccharides have been characterized according to weight using MALDI-MS, and several of the smaller macromolecules have been characterized. Lacto-N-fucopentaose I, II, and III are comprised of Gal, Fuc, GlcNAc, Gal, Glc with the isomers distinguished by the linkage between the fucose residue to the terminal galactose or penultimate GlcNAc, which may be through the second (to galactose), third or fourth (to GlcNAc) carbon. Glycoconjugates may protect the intestinal epithelium in a variety of ways. They may prevent the early events in pathogen colonization, or competitively inhibit binding of toxins such as the *E. coli* stable toxin (ST).

Oligosaccharides containing α1,2-linked fucose can inhibit ST of E.coli, are present in milk at a concentration of ~30 ppb, and can strongly inhibit ST at 30 ppb. Furthermore, glycans containing α1,2-linked fucose strongly bind to *Campylobacter jejuni*. In a study of diarrhea among nursing Mexican children, the presence of specific Lewis-ABO(H) blood group antigens in milk correlated with significant protection against diarrhea; again, oligosaccharides containing α1,2-linked fucose were protective. A likely mechanism is the inhibition of pathogen-driven processes of bacterial attachment and invasion. Experimental systems utilizing transgenic mice with human α1,2-linked fucose activity in their mammary glands showed that in infant mice consuming transgenic milk containing α1,2-linked fucose, intestinal colonization declined to 0%, while those nursing non-transgenic dams were unable to eliminate the infection. Additional experiments suggest that fucosylated glycans inhibit Norwalk virus and other human caliciviruses.

Human milk oligosaccharides seemed to protect nursing infants against diarrhea caused by ST-producing *E. coli* strains; the levels of 2-linked fucosyloligosaccharides were significantly lower in the milks consumed by symptomatic children than in milk consumed by asymptomatic and uninfected children. Low levels of 2'FL (H-2 epitope) in milk were also associated with approximately four fold higher risk of Campylobacter-mediated diarrhea; high levels of 2'FL(H-2) correlated with heightened protection.

High levels of LDFH-1 (Le^b) in milk were associated with significantly reduced incidence of norovirus diarrhea, and diarrhea from all causes was significantly lower in groups with high levels of 2-linked oligosaccharides in milk.

There is evidence for inhibitory activity by human milk glycoconjugates against *Streptococcus pneumoniae*, enteropathogenic *E. coli* (EPEC), *Campylobacter jejuni*, and Stable toxin of *E. coli* (ST). Glycopeptides appear to protect against enterohemorrhagic E. coli (EHEC), glycoprotein against rotavirus, glycosaminoglycan against Human Immunodeficiency Virus (HIV), and mucin against S-fimbriated *E. coli*.

Glycolipids associated with toxin binding are as follows: GM1 (cholera toxin, labile toxin, and the toxin of *C. jejuni*) and Gb3 (shiga toxin I and II from Shigella or *E. coli*). Sulfatide inhibits HIV and *Salmonella*-induced pathogenesis.

The human milk glycoconjugates may act as cell surface or toxin receptor homologs that absorb toxins or subvert pathogen-directed attachment and invasion. For example, expression of soluble competitors of pathogen targets may compete with epithelial binding sites, allowing unattached pathogens or toxins to be removed by peristalsis.

Follow-up research should include more detailed characterization of the inventory and activity of the high molecular weight glycans of human milk, develop methods for large scale production, and test specific glycoconjugates in a variety of systems against *Campylobacter* spp, cholera, ST, caliciviruses, and EPEC.